

Host-plant specialization in the *Drosophila melanogaster* species complex: A physiological, behavioral, and genetical analysis

(*Drosophila sechellia*/breeding sites/egg production/oviposition/adult attraction)

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ABSTRACT *Drosophila sechellia*, endemic to the Seychelles, breeds in a single resource, *Morinda citrifolia*, whereas its close sympatric relative, *Drosophila simulans*, is a cosmopolitan generalist breeding in a great variety of resources. The effects of morinda on various fitness traits of these two species, their F₁ hybrids, and reciprocal backcrosses were analyzed. Morinda fruit is highly toxic to *Drosophila* species, except *D. sechellia*. The toxicity is expressed in adults, embryos, and larvae. In embryos, early mortality is a maternally inherited trait, depending only on mother's genotype. The tolerance of *D. sechellia* to morinda is fully dominant in F₁ hybrids. Egg production is stimulated by morinda in *D. sechellia* but inhibited in *D. simulans*; in hybrids, the inhibition observed in *D. simulans* is dominant. Morinda is an oviposition attractant for *D. sechellia* but a repellent for *D. simulans*; F₁ hybrids and backcross individuals exhibit intermediate, approximately additive, behavior. In the field, adult flies of the two species exhibit opposite behavior in that *D. sechellia* is attracted to morinda and *D. simulans* is attracted to banana; hybrids have an intermediate behavior. These differences between the species explain why they do not hybridize in nature although living in sympatry. The various traits have different genetic bases: three or four different genes, or groups of genes, differentiate the ecological niches of the two species.

Ecologists have long noticed that an increase in biodiversity is accompanied by a diversification of the ecological niches and a differentiation of the resource-habitat preferences, so that coexisting species tend to avoid competition (1). For numerous insect species, the ecological niches involve some host-plant specificity that is mediated by various chemical cues (2). Differential utilization of various resources or habitats is a classical model in population genetics for the maintenance of polymorphism (3, 4). On the other hand, ecological differentiation within a population and establishment of genetic preferences (e.g., host races) appears to be a prerequisite in models of sympatric speciation (5, 6).

Most drosophilid species are saprophagous and develop in decaying plant materials, including sweet fruits, leaves, vegetables, flowers, and fungi. The diversity and specificity of breeding sites are known for numerous species (6–8), but the recognition mechanisms are generally not understood. Among the few cases analyzed at a chemical level are the cactophilic species in North American deserts (9, 10) and some mycetophilic species (11). At a population intraspecific level, various examples of microspatial variations and habitat preferences have been described (12–14), but the precise mechanisms remain unclear.

The *Drosophila melanogaster* subgroup comprises eight related Afro-tropical species for which evolutionary history is quite well understood (15–17). Examples of microspatial

variations are known in *D. melanogaster* (18, 19). On the other hand, at least two of the eight species are ecological specialists (i.e., *Drosophila erecta* on *Pandanus* and *Drosophila sechellia* on *Morinda citrifolia*). *D. sechellia* is especially interesting since it produces hybrids with some other species of the subgroup. The fertility of hybrid females between *Drosophila simulans* and *D. sechellia* has permitted preliminary genetic analyses of various traits including hybrid male sterility, the structure of male genitalia, and some morphological differences (20, 21). However, traits that are responsible for the ecological specialization of *D. sechellia* have not been investigated. In this paper, we have analyzed the physiological and behavioral traits that explain the specialization of *D. sechellia* on morinda. The results present a clear similarity with what was already known for *Drosophila pacifica* and its host plant the Senita cactus (9). In the present study, however, we investigate the genetic bases of the specialization by studying F₁ hybrids and reciprocal backcrosses between *D. sechellia* and *D. simulans*.

MATERIALS AND METHODS

We used mass cultures of *D. simulans* and *D. sechellia* collected in the Seychelles in 1985. Interspecific hybrids were produced by crossing *D. simulans* females with *D. sechellia* males, since this cross is much easier than the reciprocal one (22). Hybrid males are sterile but females are fully fertile; they were backcrossed to either parental species, producing second generation progeny, designated backcrossed (BC) *simulans* and BC *sechellia*, respectively. In toxicity experiments, a few other species, noted in the text, were also studied. All laboratory experiments were carried out at 25°C.

Morinda citrifolia (Rubiaceae) is the only host plant of *D. sechellia* (23). Ripe fruits were collected in various tropical islands (Seychelles, Mauritius, Moorea, and Martinique), brought to the laboratory, and frozen. Such material proved to be convenient for eventually analyzing the effects of morinda as food.

Physiological and behavioral traits were studied on parental species and F₁ and BC progeny. Adult survival was monitored on groups of 20 flies kept in ordinary vials with 6 g of mashed morinda. For embryonic survival, groups of 50 freshly laid eggs, aged 0–2 hr, were placed on small pieces of filter paper and transferred to mashed morinda for various periods. Egg production in the absence or presence of a small amount of morinda was studied in single pairs kept in oviposition cages (see ref. 23). We used a cornmeal-sugar medium seeded with live yeast. For measuring oviposition choice, about 10% of the food surface was covered with morinda; eggs were counted separately on and outside the morinda. Finally, the attraction of adult flies to banana or morinda resources was studied in the park of Gif during the

warmer months of the year. At least 2000 laboratory-grown flies were released in a shaded area. Traps baited either with banana or morinda were put at distances of 50, 100, and 150 m from the release point. At each site, two traps, one containing banana as bait and the other containing morinda as bait, were put in a close vicinity, 1–2 m apart, so that the adults could choose the more attractive resource. Adults in the traps were collected and identified daily.

RESULTS

Adult Survival. Several species that were found to breed in rotten morinda either in the Seychelles or in Mauritius (refs. 23 and 24 and unpublished observations) were tested with morinda. Survival curves (Fig. 1A) demonstrate the high toxicity of morinda for all these species. A large mortality, >50% of the total, was often observed during the first hour of the treatment. *D. melanogaster* was a little more tolerant than the others. In all cases, all flies were dead after 7 days. Results for *D. sechellia*, *D. simulans*, and hybrid progeny are shown in Fig. 1B. *D. simulans* adults were rapidly killed, as were the other species. By contrast, >90% of *D. sechellia* adults were alive after 8 days, and larvae were breeding in the resource. F₁ hybrid adults were clearly similar to their *D. sechellia* parent, as well as the BC *sechellia* flies. Only the BC *simulans* adults exhibited an intermediate survival curve. These observations suggest that the high tolerance of *D. sechellia* to the toxic effects of morinda behaves as a dominant genetic trait in the F₁ progeny.

Embryonic Survival. Freshly laid eggs, <2 hr old, were put in contact with morinda for a period of 5–60 min and then

transferred to normal food. Egg hatching was observed after 24 hr. Results are given in Fig. 2. In *D. simulans*, the contact with morinda increased the mortality so that after ≈10 min 50% of the embryos were killed and after 30 min >90% of the embryos were killed. In *D. sechellia*, a lower hatchability was observed in the controls without contact with morinda, which is usual in this species (23). However, no significant increase was found after the morinda treatment. Even permanent contact for >20 hr did not prevent hatching. Hybrid F₁ embryos, laid by *D. simulans* females that were mated with *D. sechellia* males, exhibited the same sensitivity as pure *D. simulans* embryos. Hence, the early embryonic mortality appears to be a maternally inherited trait, not influenced by the genotype of the embryo.

F₁ females were mated to either parental species. Egg hatchability was much better in the matings with *D. simulans* males, as compared with *D. sechellia* males. On the other hand, there was no decrease of egg hatching due to morinda toxicity. Again, embryonic sensitivity seemed determined by the genotype of the mother only. F₁ mothers produced embryos that were insensitive to morinda toxins; the *D. sechellia* characteristics were dominant in F₁ hybrids. When embryos from BC *simulans* females were studied, a toxic effect of morinda was observed that was less pronounced than with pure *D. simulans* embryos (i.e., 50% of the embryos were killed by 30 min).

Early embryonic mortality appears to be a maternal trait depending on the mother's genotype. On the other hand, F₁ females were resistant to morinda and BC *simulans* females exhibited intermediate properties. These results are similar to those observed for adult survival, especially the complete

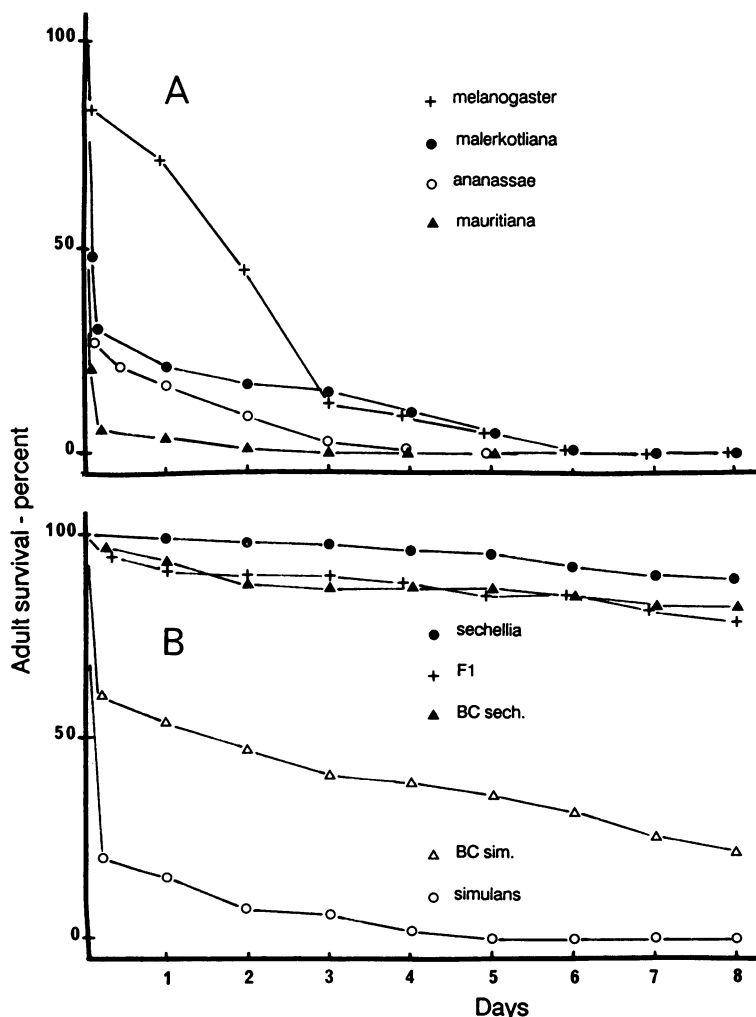


FIG. 1. Survival curves of adults in vials with 8 g of fresh morinda. (A) Survival of four sensitive species of the *D. melanogaster* group. (B) Survival of *D. sechellia*, *D. simulans*, F₁ hybrids, and backcross individuals. For each curve, six groups of 20 flies were studied. There was no significant difference between sexes. BC *sech.*, BC *sechellia*; BC *sim.*, BC *simulans*.

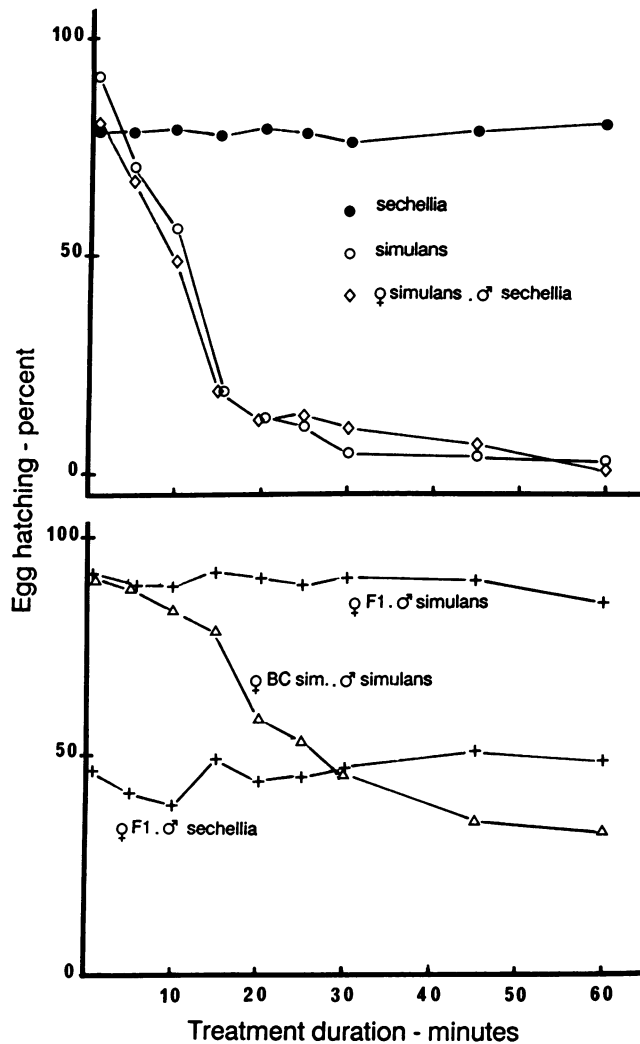


FIG. 2. Survival curves of young embryos kept in contact with fresh morinda between 0 and 60 min. For each point, at least 250 eggs were studied. BC sim., BC *simulans*.

dominance in the F₁ hybrids. We might expect that the maternal effect would be valid in young embryos only. When

older embryos (18 hr) were submitted to morinda, even the *D. simulans* embryos were able to hatch and no further analysis was possible. The presence of the larval tegument could be sufficient to protect the embryos.

Egg Production. We confirmed the stimulation of egg production by morinda in *D. sechellia* (23) but found an inhibition in *D. simulans*. Results for parents and hybrid offspring are given in Table 1.

In the parental species, the difference in fecundity was mainly due to a large difference in the ovariole numbers of the ovaries. In addition, the maximum rate of egg production per ovariole was 2 eggs per day in *D. simulans* but only 1.1 eggs per day in *D. sechellia*. When 500 mg of morinda was present on the food, the rates became identical (1.5) due to a stimulation in *D. sechellia* and an inhibition in *D. simulans*. In F₁ females, the ovariole number was intermediate between the parents. The rate of egg production on normal food was similar to that of *D. simulans*, and the addition of morinda resulted in an inhibitory effect. In this case, the characteristics of the *D. simulans* parent were obviously dominant. In the backcrossed flies, coherent data were obtained: BC *simulans* females exhibited *D. simulans*-like characteristics; BC *sechellia* females had an intermediate rate of egg production in the absence of morinda and were insensitive to the presence of this fruit.

The morinda fruit has a strong smell and we investigated whether the fragrant volatile products would be sufficient to modify the rate of egg production. Special oviposition cages were constructed in which the morinda was not accessible to the flies because of a wire screen. The change in egg production over 10 days due to the presence of morinda was -9.0 ± 88.8 eggs in *D. simulans* and $+6.4 \pm 9.6$ eggs in *D. sechellia*. These differences are in the same direction as those observed in Table 1 but are much lower and not significant. Apparently, a direct access to the morinda is necessary for getting strong stimulation or inhibitory effects.

Oviposition Choice. About 10% of the food surface was covered by a small amount of morinda (50 mg). Eggs laid either on morinda or next to it were counted daily and cumulated over successive days. The mean proportions of eggs laid on morinda are shown in Fig. 3. There is a clear attraction for the morinda in *D. sechellia* females and a repulsion for *D. simulans* females. F₁ females are intermediate between parents but still exhibit some preference for

Table 1. Influence of morinda on egg production

Genotype	Morinda	n	Ovarioles, no.	Egg production		
				10 days	Max	Rate
<i>D. sechellia</i>	Yes	9	18.0 ± 0.6	198.3 ± 9.1	26.8 ± 1.1	1.52 ± 0.07
	No	15	18.5 ± 1.0	149.8 ± 5.8	20.1 ± 0.8	1.11 ± 0.06
	Difference		-0.5 ± 1.4	+48.5 ± 10.2**	+6.7 ± 1.4**	+0.41 ± 0.10**
<i>D. simulans</i>	Yes	9	35.6 ± 0.4	453.1 ± 16.1	54.3 ± 2.5	1.53 ± 0.07
	No	14	35.9 ± 0.6	602.1 ± 21.7	72.7 ± 2.8	2.04 ± 0.07
	Difference		-0.3 ± 0.7	-149.0 ± 28.5**	-18.4 ± 4.0**	-0.51 ± 0.1**
F ₁ hybrid	Yes	10	27.8 ± 0.7	362.1 ± 15.6	44.8 ± 1.8	1.62 ± 0.07
	No	14	27.9 ± 0.5	457.9 ± 9.9	58.5 ± 1.2	2.11 ± 0.06
	Difference		-0.1 ± 0.8	-95.8 ± 17.7**	-13.7 ± 2.0**	-0.49 ± 0.09**
BC <i>simulans</i>	Yes	14	31.1 ± 0.9	390.1 ± 10.3	51.6 ± 1.4	1.67 ± 0.07
	No	15	30.6 ± 0.7	486.8 ± 12.0	63.0 ± 1.6	2.07 ± 0.05
	Difference		0.5 ± 1.1	-96.7 ± 15.9**	-11.4 ± 2.1**	-0.40 ± 0.09**
BC <i>sechellia</i>	Yes	11	22.2 ± 0.7	197.3 ± 20.7	27.8 ± 3.1	1.27 ± 0.14
	No	13	21.5 ± 0.5	200.0 ± 15.8	28.1 ± 2.3	1.30 ± 0.10
	Difference		0.7 ± 0.8	-2.7 ± 25.6	-0.3 ± 0.1	-0.03 ± 0.16

Influence of morinda (500 mg) on egg production traits in *D. sechellia*, *D. simulans*, F₁, and backcross females. n, Number of females studied. Egg production was measured as follows: 10 days, total egg production during the first 10 days; Max, maximum daily egg production (mean of day 4 to day 8); rate, rate of egg production per ovariole per day (ratio of Max. to the ovarioles number). Values are mean ± SEM; comparisons are made with Student's *t* test. Significance levels are *P* < 0.05 (*) or *P* < 0.01 (**).

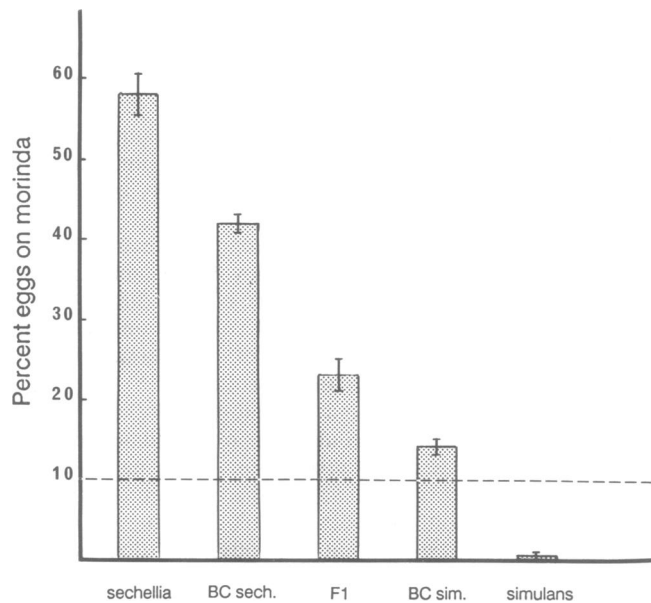


FIG. 3. Oviposition choice in parental species, F₁ hybrids, and BC individuals. For each genotype, 10 groups of flies were studied. Error bars indicate the standard error of the mean percent. The 10% line corresponds to an absence of choice. BC sech., BC *sechellia*; BC sim., BC *simulans*.

morinda. Females from backcrosses again are intermediate between F₁ hybrids and parents. Thus these results suggest an additive genetic basis for oviposition choice.

Resource Selection in Natural Conditions. The results of recapture in nature are shown in Fig. 4. Almost all *D. sechellia* adults (98.4%) were collected in morinda traps, whereas *D. simulans* preferred banana (91%). F₁ hybrids exhibited an intermediate behavior with a small preference for banana (64.1%). Finally, the backcrossed individuals were, in both cases, close to the parental species to which F₁ females were crossed.

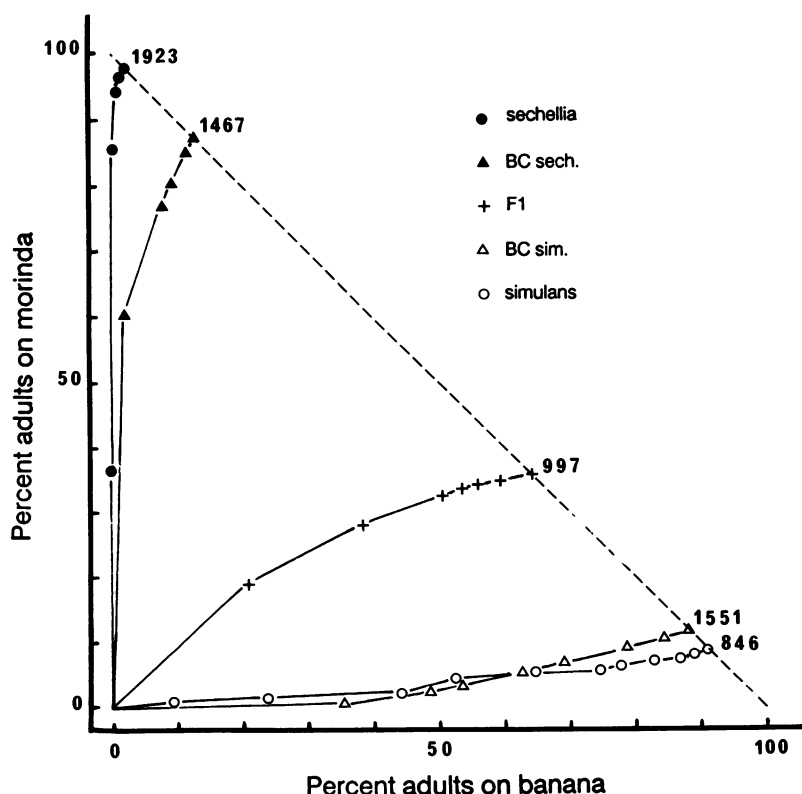


FIG. 4. Results of five recapture experiments in natural conditions. Released adults were attracted by neighboring traps containing either morinda or banana. Total numbers of collected flies on both resources are given for each genotype. Data of successive days of collection are expressed as the cumulated percentages of the total number. Experiments with parental species and F₁ hybrids were repeated twice, with very similar data. BC sech., BC *sechellia*; BC sim., BC *simulans*.

DISCUSSION

Various physiological and ethological traits explain the specialization of *D. sechellia* on a single resource. The most conspicuous observation is the high toxicity of fresh morinda for presumably all *Drosophila* species except *D. sechellia*. Even this species is not completely tolerant to the toxic products in the ripe fruit. For example, when *D. sechellia* eggs were deposited on fresh morinda, the eggs hatched but most of the larvae died during development. In natural conditions, and especially in places where *D. sechellia* does not exist, various *Drosophila* species emerge from fallen rotten morinda fruits (ref. 24 and unpublished results) because microorganisms in the fruit detoxify the toxic products after a few days. The tolerance of *D. sechellia* provides a temporal advantage and allows an early colonization of the resource. This may explain why *D. sechellia* still exists in the Seychelles in competition with *Drosophila malerkotliana*, in spite of its low reproductive potential and poor competitive ability on nontoxic food (23).

Other genetically determined traits have evolved and strengthened the relationship between *D. sechellia* and its host plant. In particular, *D. sechellia* adults quickly detect their resource over a long distance, >150 m. An almost complete preference is shown when a choice is possible, whereas *D. simulans*, as presumably all other *Drosophila* species, exhibits the opposite behavior. This capacity of *D. sechellia* has clear ecological significance, since morinda trees may be dispersed. Fruits are not produced permanently by the same tree, and finding ripe fruits from one patch to the other is a must for survival. When the host plant is discovered, at least two other traits increase the fitness of *D. sechellia*. (i) The females exhibit a short distance oviposition preference for morinda, whereas *D. simulans* is repelled. (ii) In the presence of morinda, the oogenesis of *D. sechellia* females is specifically stimulated, increasing the number of progeny.

These various adaptations of *D. sechellia*, which do not exist in the closely related *D. simulans*, raise two questions.

(i) What are the chemicals in morinda? (ii) What are the genes responsible for the divergence of the two related *Drosophila* species? Extraction and purification of morinda products have been undertaken. Available observations suggest that there are at least two kinds of chemicals. (i) Toxic products are responsible for the death of adults, larvae, and embryos of *Drosophila* species. (ii) Fragrant volatiles are responsible for the typical smell of morinda fruit and for the long-distance attraction of adults. Other compounds could also be responsible for other effects. For example, the oviposition preference for morinda and the stimulation of egg production were not elicited by an olfactory perception of the volatile compounds. It seems unlikely that such favorable effects could be induced by the toxic compounds.

The partial genetic analysis made by studying F_1 hybrids and backcrosses helps to discriminate the various traits. For the tolerance, we found complete dominance of the *D. sechellia* characteristics in F_1 hybrids. The properties of the *D. simulans* parent are dominant with respect to the rate of egg production on normal food or its inhibition due to morinda. Finally, near additivity exists for the behavioral traits of oviposition choice and adult attraction. Even if a single locus with different alleles in the parent species is responsible for each genetic difference, we must conclude that the specialization of *D. sechellia* on morinda involves a genetic change of at least three or four independent loci.

When comparing two related species, it is generally not possible to decide which one exhibits the ancestral traits. However, since other *Drosophila* species are sensitive to the toxic compounds of morinda and not attracted by this fruit, we may consider them as an outgroup. In other words, the genetic singularities that enable *D. sechellia* to use morinda have evolved from an ancestor that had approximately the same characteristics as the present *D. simulans*. A plausible evolutionary scenario is that, on its arrival in the Seychelles, the ancestor used a diversity of resources, among which was aged, rotten, nontoxic morinda. The main difficulty was to overcome the toxicity of the fresh resource. Any mutation conferring some resistance would be favored by natural selection and would go on to be fixed. This would not, however, change the generalist ecological status of the species. As pointed out many times (5, 6, 25), a move toward specialization is likely to imply behavioral changes. In this respect, the appearance of a variant showing some preference for the morinda smell would be a crucial step toward an ecologically isolated population. Whether tolerance and preference appeared simultaneously or successively and, in the latter case, in which order remains a matter of speculation. The other adaptative traits that more strongly link *D. sechellia* to its resource (i.e., oviposition preference and oogenesis stimulation) are less important in its life history strategy and presumably evolved more recently.

When *D. sechellia* was discovered (26), it was collected only on small islands (i.e., Praslin and nearby islets), whereas *D. simulans* was found only on the big island of Mahé. They were thus allopatric. There is now evidence (ref. 27 and unpublished data) that *D. sechellia* exists also on Mahé, so that the two species live in sympatry. When we bred the two species in a population room, offering them different resources (i.e., banana and morinda), over about 6 months and 12 generations of coexistence, only 2 hybrid males were found among >2500 males examined. This prezygotic isolation seems to be due to the divergent ecological preferences.

The present coexistence of the two species in nearby sites could represent sympatric speciation. However, another scenario remains possible that corresponds to two successive colonizations of the Seychelles. The first colonizer adapted to morinda and gradually evolved into the present *D. sechellia*. Later a second colonization occurred and produced the

present *D. simulans* population, which has remained a generalist species. In favor of this two-foundation hypothesis are the observations that the Seychellian *D. simulans* population is genetically close to the populations found in other parts of the world (28, 29), whereas *D. sechellia* is more distinct (27). However, comparison of the mitochondrial genomes led to a somewhat different conclusion (30). The mitochondrial DNA of *D. sechellia* and of the Seychellian race of *D. simulans* are related, although distinct, and are separated from those found in the two other mitochondrial races of *D. simulans*.

A final point concerns the number of genes, or groups of genes, that have diverged during the speciation process and now distinguish the species. Studies of genes responsible for hybrid sterility or for morphological differences have led to the provisional conclusion that male sterility genes tend to accumulate on the X chromosome and to be recessive, whereas genes responsible for the morphological and behavioral traits are dispersed on the various chromosomes and exhibit mostly additive relationships (31). Our results do not confirm the additivity rule since, among four adaptative traits, two exhibit a complete F_1 dominance. Further investigations and more precise genetic analyses are needed of these clearly adaptative traits.

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